PRESENCE OF A HCO_3^--ACTIVATED ATPase IN PANCREATIC ISLETS

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1. Introduction

The generation and utilization of high-energy phosphates in pancreatic islets is thought to represent a critical feature in the functional behaviour of this fuel-sensor organ [1]. The consumption of O_2 by isolated pancreatic islets is markedly reduced when they are exposed to a HCO_3^--free medium, suggesting that HCO_3^- may be involved in the regulation of ATP-consuming processes [2]. This study reveals the presence of a HCO_3^--activated ATPase in rat pancreatic islets.

2. Materials and methods

Pancreatic islets isolated [3] from fed female albino rats were homogenized (Ultrasonic Disintegrator; Crawley, England; MSE, medium power, amplitude 3; twice 5 s) in groups of 300 islets each in 1.0 ml of a solution of Tris (10 mM) buffered with HCl to pH 7.5. ATPase activity [4] was measured over 5 min incubation at room temperature after addition of the islet homogenate (50 μl) to a reaction mixture (60 μl) containing (final concentration) Tris (25 mM; pH 7.5), Mg-ATP (5 mM) and, when required, the Na^+ salt of HCO_3^-, Cl^-, SO_4^{2-} or $CH_3CO_2^-$ (10 mM). The reaction was stopped by addition of iced perchloric acid (2.5 N; 25 μl). The assay tubes were then placed on ice. After neutralization with KOH (2.5 N; 30 μl) and centrifugation, an aliquot of the supernatant solution ($\geqslant$60 μl) was mixed with 2.5 ml of a mixture of $(NH_4)_6Mo_7O_{24} \cdot 2\,H_2O$ (4.2%, w/v) in 5 N HCl (1 vol.) and malachite green (0.2%, w/v) in H_2O (3 vol.). After 1 min, the coloration of this mixture was measured by spectrophotometry at 660 nm [5].

In order to estimate the contribution of mitochondria to the enzyme activity, a group of 300 islets was homogenized (manual homogenization in a tissue grinder) in 0.5 ml of a Tris solution (10 mM; pH 7.5) containing sucrose (300 mM). After centrifugation for 10 min at 400 $\times$ g to deposit intact cells, nuclei and cell debris, a mitochondrial pellet was separated by centrifugation for 10 min at 10 300 $\times$ g [6]. Enzyme activity was measured in the mitochondrial pellet (resuspended in 0.5 ml of the Tris solution) and the corresponding supernatant fraction, both being sonicated as above.

Both the control and HCO_3^--activated reactions occurred at a constant rate for $\geqslant$5 min and were proportional to the amount of tissue, the mean value found with different volumes of the same homogenate (10, 20 and 50 μl) and two different lengths of incubation (2.5 and 5.0 min) averaging 104.6 ± 9.2 and 207.3 ± 8.1 $pmol.min^{-1}.islets^{-1}$ (n = 4–5) in the absence and presence of HCO_3^- (10.0 mM), respectively. As in other tissues [7], the velocity of the reaction in the absence of HCO_3^- was tightly dependent on the pH, increasing from 33 to 97 and 187 $pmol.min^{-1}.islet^{-1}$ as the pH of the reaction mixture was increased from 7.0 to 7.5 and 8.0. Therefore, great care was taken to insure that the addition of HCO_3^- did not affect the pH of the reaction mixture. All determinations were made at pH 7.5, which is optimal for characterization of the HCO_3^--activated ATPase [7].

All measurements were made in duplicate. Whether in the absence or presence of HCO_3^-, no ATPase activity (4 ± 7 $pmol.min^{-1}.islet^{-1}$; n = 4) was found

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in the absence of ATP, or in the presence of ATP using a boiled islet homogenate. All data were corrected for the blank value found under the same experimental conditions in the absence of homogenate. Such a blank value amounted to <15% of the reference value for HCO_3^--activated ATPase activity, and was proportional to the ATP concentration of which it represented ~1–2%. Results were expressed as pmol P_i formed.min^{-1}.$islet^{-1}$, by reference to appropriate standards (KH_2PO_4; 5–25 nmol). Mean values (± SEM) are given together with the number of individual determinations (n). Control values refer to the activity found in media deprived of HCO_3^- (with or without NaCl 10 mM).

3. Results

The ATPase activity averaged 144 ± 16 and 267 ± 17 pmol.min^{-1}.$islet^{-1}$ (n = 10 in each case; P <0.001) in the presence of NaCl and $NaHCO_3$ (10 mM), respectively. The HCO_3^--induced change in activity corresponded to a 88 ± 16% increment above the paired control value. No stimulation of ATPase activity was found with either Cl^-, SO_4^{2-} or $CH_3CO_2^-$ (10 mM each), the velocity of the reaction in the presence of these anions averaging 94.2 ± 12.4%

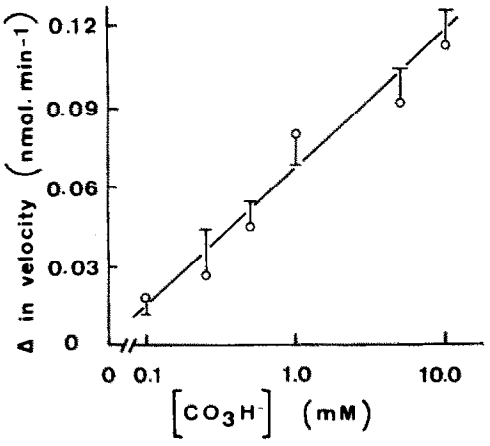

Fig.1. Effect of HCO_3^- upon ATPase activity in islet homogenates. The increase in reaction velocity attributable to HCO_3^- is shown as a function of the HCO_3^- concentration (logarithmic scale). Mean values (± SEM) are expressed as nmol.min^{-1}.$islet^{-1}$ and refer to 3–4 individual measurements.

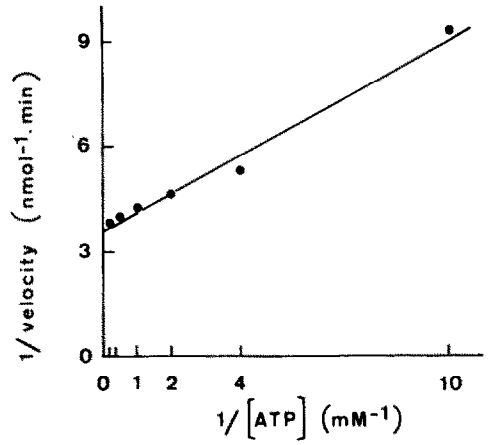

Fig.2. Double reciprocal plot for HCO_3^--activated ATPase activity in islet homogenates. Mean values for reaction velocity are expressed as nmol.min^{-1}.$islet^{-1}$ and refer to 2 individual experiments.

(n = 4) of the paired control value. The magnitude of the HCO_3^--induced increase in reaction velocity was related to the HCO_3^- concentration (0.1–10.0 mM) in a saturable manner. There was a tight correlation (r = 0.867; n = 19; P <0.001) between the magnitude of such an increase and the log of HCO_3^- concentration, with a half-maximal value at HCO_3^- 0.6–0.8 mM (fig.1). The K_m of the HCO_3^--activated ATPase for ATP amounted to 0.15 mM, with a V_{max} close to 278 pmol.min.$islet^{-1}$ (fig.2). The HCO_3^--activated ATPase activity was recovered in both a mitochondria-rich pellet (36%) and the corresponding supernatant fraction (64%). The ratio of HCO_3^--stimulated to control ATPase activity was similar in the whole homogenate (1.93), mitochondrial pellet (1.85) and supernatant fraction (1.81).

4. Discussion

A HCO_3^--activated ATPase was found in several gastrointestinal organs, such as frog and dog gastric mucosa [7,8], and dog and cat (exocrine) pancreas [9]. This enzyme was also studied in rat liver mitochondria [4,10]. As a rule, HCO_3^- causes a 2-fold increase in reaction velocity. Relative to the protein content of the tissue sample (~0.8 μg protein/islet; see [11]), the activity in the pancreatic islets is of the same order of magnitude as that found in gastric

mucosa or exocrine pancreas [7,9]. In the latter tissues, it was postulated that the HCO_3^--activated ATPase participates in active HCO_3^- secretion [7–9]. The physiological significance for such an enzyme in pancreatic islets is open to speculation. It is noteworthy, however, that the range of concentrations ($\leqslant$3 mM) in which HCO_3^- exerts its most pronounced stimulant action on ATPase activity coincides with the range of concentrations in which extracellular HCO_3^- affects O_2 consumption [2] and insulin release [12] by the islets. This analogy raises the idea that a membrane-associated HCO_3^- activated ATPase might participate in the regulation of ATP utilization by pancreatic islet cells.

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